THE EFFECTS OF CALCIUM ON ISOLATED ARTHROPOD MUSCLE FIBERS [1]

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The protoplasm of a muscle fiber is normally in a fluid state. This fluidity of muscle protoplasm has been demonstrated in frog muscle by Rieser (1949). Moreover, the protoplasm of muscle like that of other types of cells can and does undergo a gelation—similar to the clotting of blood. For a description of the clotting reaction see Speidel (1938); Heilbrunn and Wiercinski (1947); Woodward (1948). In marine eggs and protozoa, calcium and thrombin-like substances can cause protoplasm to clot (Heilbrunn and Daugherty, 1932). It is believed that the colloidal system of muscle is essentially similar to the colloidal system of marine eggs and protozoa and, in many respects, is similar to the colloidal system of blood. In an isolated cut muscle fiber the normally fluid protoplasm undergoes a gelation at the cut end of the fiber and this gelation progresses along the fiber (Heilbrunn, 1940). Here then is a system which can be studied. Woodward (1948) has studied the clotting properties of the vertebrate muscle. With few exceptions (for example, see Yaeger and Hager, 1934; Proctor, 1952) there is no available information concerning the effects of calcium on isolated invertebrate muscle fibers. The present study was performed on muscle fibers of invertebrates to determine: 1) whether or not the gelation of the muscle protoplasm is influenced by the calcium ion; 2) to compare the rate of clotting of invertebrate fibers with the rate of clotting of vertebrate fibers; and 3) to determine whether or not the clotting reaction can be inhibited.

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Materials and Methods

Various types of arthropods, both terrestrial and marine, were used in the present study of isolated single muscle fibers. The marine forms, which were obtained at the Marine Biological Laboratory (Woods Hole, Mass.), were *Callinectes sapidus* (blue crab) and *Homarus americanus* (lobster). The terrestrial arthropod used mainly in the experiments reported here was *Schistocerca americana* (grasshopper).

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Artificial Ca-free sea water served as the immersion medium for the marine arthropod studies. This modification of McClendon's artificial sea water was made up as follows: 490 cc. of 1 *M* NaCl, 10 cc. of 1 *M* KCl, 30 cc. of 1 *M* $MgCl_2 \cdot 6H_2O$, 20 cc. of 1 *M* $MgSO_4$ and distilled water to make a liter of solution. The pH of this solution varied from 7.8–8.6.

Bělař's (1929) insect Ringer, with calcium chloride omitted, was used as the dissecting medium for studies on the grasshopper.

Merck's reagent grade chemicals and glass (Pyrex) doubly distilled water were used exclusively in the preparation of all solutions.

All pH measurements were made with a glass electrode (Beckman) pH meter. Where necessary, pH adjustments were made with small quantities of 0.1 N NaOH or 0.1 N HCl.

A molar solution of calcium chloride was used as a stock solution and this was diluted with Ca-free Ringer to the desired concentration.

The femur of the large jumping leg (metathoracic) of the grasshopper was removed by cutting just distal to the coxotrochanter and just proximal to the femoral-tibial joint. The chitin was slit both along the anterior and the posterior part of the femur. This reveals the large plume-shaped muscles attached by their relatively large fibers to the chitin of the femur at one end and to a broad flat central tendon at the other. The chitin, which is now in two halves, can be removed by carefully pulling it in opposite directions from the distal end. This process leaves the entire muscle attached to one half of the cuticle. By grasping the muscle by its tendon at its distal end, the entire muscle may be peeled from the cuticle. This muscle appears relatively large, composed of numerous fibers varying in length (1.5–4.0 mm.) and diameter (0.15–0.29 mm.). The entire muscle was placed immediately in Bělař's (Ca-free) insect Ringer and single fibers were isolated by transverse cuts with steel dissecting needles, as close to the tendon as possible and cutting through the numerous tracheoles which bind the fibers.

The ends of the grasshopper muscle fibers, which are fastened to the chitinous exoskeleton, are covered with a brown or white pigment (depending upon the species). This pigmented end does not appear to be very permeable. Thus, the preparation essentially consists of a single muscle fiber each with one cut end.

After transferring a fiber to a depression slide with the aid of a pipette, the Ca-free insect Ringer was replaced with a test solution. A fresh cut was made across the fiber and the effects of the test solution were observed immediately. Some authors (see Woodward, 1948) rinse the fibers with two or three changes of the test solution. However, since all the fibers in these experiments were isolated in Ca-free insect Ringer, this technique was not thought necessary.

The clotted portion of the fiber was measured, at varying intervals (30 seconds–30 minutes) after cutting, under a magnification of approximately 43 ×, by means of a Ramsden-type filar micrometer eyepiece (Bausch and Lomb with 12.5 × eyepiece).

Muscle fibers of marine forms (crab and lobster) were isolated by cutting off the walking legs, removing the chitinous covering, and dissecting out single fibers from the exposed muscles in Ca-free sea water. In no cases were fibers used experimentally more than 45 minutes after amputation of the leg from the animal.

Results

Under the influence of calcium two visible changes occur in isolated single muscle fibers of arthropods: 1) a shortening of the fiber, and 2) the formation of a clot. Although these two phenomena occur concurrently in invertebrate muscle fibers, they will be considered separately in this paper.

1. Shortening

a. Blue crab. Single muscle fibers from the blue crab (*C. sapidus*), which had been isolated in Ca-free sea water, were placed immediately in isotonic calcium chloride solution (0.33 *M*). In each case, a fresh cut was made across the fiber and the length of the cut single fiber was then measured. As indicated by Tables I and II, these pieces of isolated muscle fiber varied in their original length. Following exposure to calcium the fibers shorten. The degree of shortening of each fiber was determined by the product of the final length times 100, divided by the

Table I

Effects of isotonic $CaCl_2$ (0.33 M) pH 5.7–6.0 (single muscle fibers)

Blue Crab (*Callinectes sapidus*)

Original length mm.	Time min.	Rate of clotting mm./min.	Final length mm.	% Shortened
3.519	1.0	0.247	1.711	51.3
2.608	1.0	0.219	1.556	59.6
3.651	1.0	0.293	2.934	19.7
3.995	1.0	0.472	1.980	50.5
3.504	1.0	0.446	1.801	48.7
3.463	1.0	0.352	2.078	40.0
3.569	1.0	0.802	2.475	30.4
3.341	1.0	0.207	1.548	53.7
3.260	1.0	0.393	1.483	54.5
5.623	1.0	0.262	2.863	48.6
3.260	1.0	0.328	2.773	14.0
2.950	0.75	0.958	2.078	29.6
3.039	1.0	0.413	1.752	42.4
3.260	1.0	0.195	2.387	26.8
2.542	0.5	1.03	1.418	44.3
2.852	1.0	0.250	1.344	52.9
2.811	0.25	0.995	1.956	30.5
2.991	1.0	0.351	1.589	49.6
3.121	1.0	0.487	2.094	33.0
2.469	1.0	0.387	1.548	37.3
3.285	1.0	0.359	2.135	34.1
4.792	1.0	0.328	1.996	58.4
3.537	1.0	0.767	2.942	16.9
		0.461		40.3

TABLE II

Effects of isotonic $CaCl_2$ (0.33 M) pH 8.0–8.2 (single muscle fibers)
Blue Crab (*Callinectes sapidus*)

Original length mm.	Time min.	Rate of clotting mm./min.	Final length mm.	% Shortened
4.360	1.0	0.588	1.654	62
3.243	1.0	—	1.670	48.5
5.321	1.0	0.608	1.956	63.3
5.599	1.0	0.328	3.553	36.6
5.028	1.0	0.467	3.088	38.6
3.349	1.0	0.640	2.326	31.2
8.581	1.0	0.452	4.360	49.2
3.594	0.5	0.502	1.793	50.2
7.359	1.0	0.502	2.591	64.8
7.726	1.0	0.410	5.028	35.0
8.655	1.0	0.443	5.868	32.3
6.414	1.0	0.561	3.742	41.7
6.715	2.0	0.294	4.319	35.7
		0.482		45.3

original length. This value when subtracted from 100 gives the per cent of shortening. This may be stated:

$$\text{per cent of shortening (p. s.)} = 100 - \left(\frac{\text{final length}}{\text{original length}} \times 100\right)$$

The shortening of fibers exposed to 0.33 *M* $CaCl_2$ (pH 5.7 to 6.0) varied from 14% to 59% (Table I). The average per cent shortening was about 40%. The pH of this solution (5.7–6.0) was lower than that of normal sea water. When isolated blue crab muscle fibers were exposed to 0.33 *M* $CaCl_2$ at the normal pH of sea water (8.0–8.2), the average shortening was 45.3% (Table II).

b. Lobster. Twelve fibers from the lobster (*H. americanus*), immersed in 0.33 *M* $CaCl_2$, showed an average shortening of 33.7%.

c. Grasshopper. The fibers of the grasshopper (*S. americana*) were extremely sensitive to calcium. Twenty-three fibers of this arthropod showed an average shortening of 58% in a solution containing calcium chloride in a concentration of 0.0018 *M*.

2. Clotting

The second visible change occurring in isolated single muscle fibers, as a result of the action of the calcium ion, is the formation of a clot. The clotted protoplasm appears only at the cut end or other point of injury of the muscle fiber. The area of the clot appears as a swollen region darker in color than the remaining protoplasm. This clot moves as a wave along the muscle fiber. In addition, the clotted portion is characterized by a loss of cross striations.

a. Rate of clotting.

1) Blue crab and lobster. Data were gathered at one-minute intervals to determine the rate of movement of the protoplasmic clot in isolated single muscle fibers of the blue crab under the influence of various concentrations of calcium. The effects of four different concentrations on blue crab (*C. sapidus*) are summarized in Figure 1. In all concentrations of calcium observed, there was a rela-

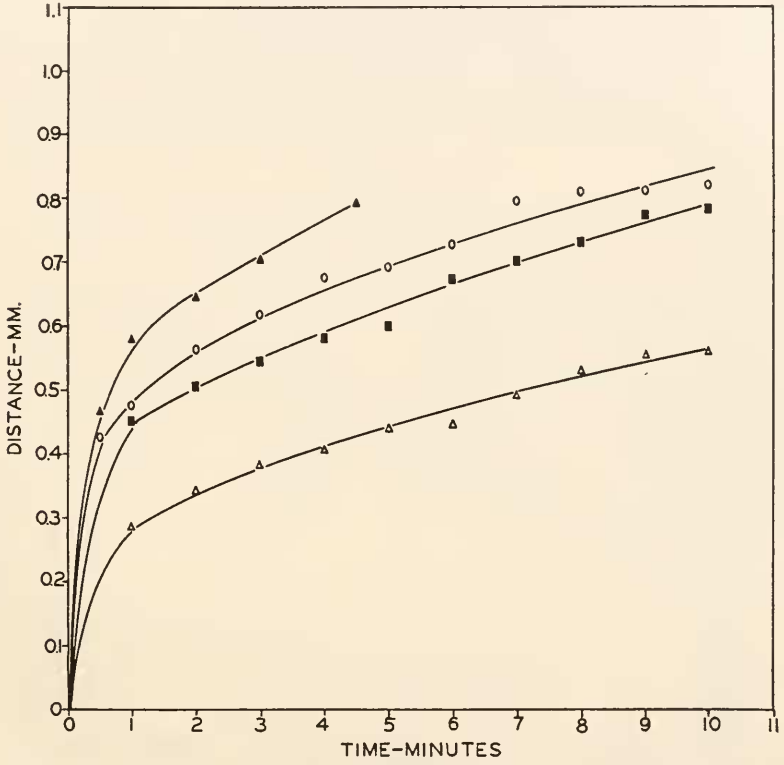

FIGURE 1. The effects of calcium on single muscle fibers of the blue crab (*Callinectes sapidus*). Legend: Open triangles (△), artificial sea water, pH 7.8, average of 10 fibers; solid squares (■), 0.083 *M* $CaCl_2$ in artificial Ca-free sea water, pH 8.2, average of 5 fibers; open circles (○), 0.165 *M* $CaCl_2$ in artificial Ca-free sea water, pH 7.5, average of 13 fibers; solid triangle (▲), 0.330 *M* $CaCl_2$ (pure), pH 6.89, average of 10 fibers.

tively high rate of movement of the protoplasmic clot of the muscle fibers during the first minute of exposure. However, this rate was not maintained at this high level for the entire period of exposure of these muscles in calcium. In Figure 1 the ordinates show the length of the clotted material in the fiber and the abscissae the time. The rates at which the clotted portion of the fiber increases in length are given by the slopes of the curves. In artificial sea water (0.0117 *M* $CaCl_2$), the average rate of movement of the protoplasmic clot of 10 fibers was 0.287 mm./min. at one minute and decreased to a rate of 0.108 mm./min. at 4 minutes (see Fig. 1). The average rate of movement of the protoplasmic clot of 5 fibers exposed

to 0.083 *M* $CaCl_2$ was 0.451 mm./min. at one minute and decreased to 0.075 mm./min. at 10 minutes. Thirteen fibers exposed to a concentration of 0.165 *M* $CaCl_2$ showed an average rate of clotting of 0.475 mm./min. at one minute and 0.0824 mm./min. at 10 minutes. Isotonic calcium chloride solution (0.33 *M*) gave a value (average of 10 fibers) of 0.534 mm./min. at one minute and 0.176 mm./min. at 4.5 minutes (see Fig. 1).

Attempts to obtain single muscle fibers from the lobster (*H. americanus*) suitable for a study of the rate of clotting were unsuccessful. These fibers showed an inherent tendency to stick together. This "stickiness" of the fibers caused them to adhere to the dissecting needles, thus causing injury.

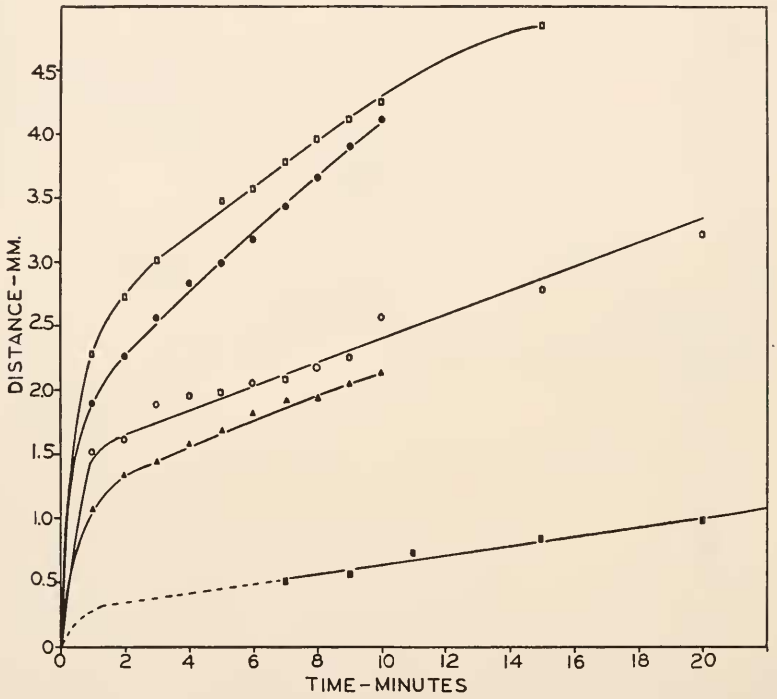

FIGURE 2. The effects of calcium on single muscle fibers of the grasshopper (*Schistocerca americana*). Legend: Open squares (□), 0.0175 *M* $CaCl_2$ in Ca-free Ringer, pH 7.25, average of three fibers; solid circles (●), 0.0087 *M* $CaCl_2$ in Ca-free ringer, pH 7.22, average of three fibers; open circles (○), 0.0044 *M* $CaCl_2$ in Ca-free Ringer, pH 7.32, average of 6 fibers; solid triangles (▲), 0.0018 *M* $CaCl_2$ in Ca-free Ringer, pH 7.5, average of 9 fibers; solid squares (■), Ca-free Ringer, pH 7.3, average of 5 fibers.

2) Grasshopper. Data were gathered at one-minute intervals to determine the rate of clotting of protoplasm in various concentrations of calcium. The wave-like movement of the clot along the muscle fiber showed considerable variation in speed even in fibers from the same animal.

Figure 2 summarizes the results of the effects of various concentrations of calcium on single muscle fibers of the grasshopper (*S. americana*). The ordinates show the length of the clotted material in the fiber, the abscissae the time. The

rates at which the clotted material increases in length are given by the slopes of the curves. Four concentrations of calcium chloride were used in these experiments (see Fig. 2) : 0.0175 *M* (top curve, open squares) ; 0.0087 *M* (solid circles) ; 0.0044 *M* (open circles) ; and 0.0018 *M* (solid triangles). The pH of these test solutions varied from 7.22–7.50. Muscle fibers exposed to these four test solutions show a relatively high rate of movement of the protoplasmic clot during the first minute of exposure. The effects of various concentrations of calcium on the rate of protoplasmic clotting of single muscle fibers during the first minute are as follows: fibers immersed in 0.0175 *M* $CaCl_2$ showed an average rate of clotting of 2.297 mm./min.; fibers immersed in 0.0087 *M* $CaCl_2$ had an average clotting rate of 1.899 mm./min.; fibers immersed in 0.0044 *M* $CaCl_2$ showed an average clotting rate of 1.512 mm./min.; and those immersed in 0.0018 *M* $CaCl_2$ (the calcium con-

TABLE III

Effects of calcium on rate of protoplasmic clotting (single muscle fibers)

Grasshopper (*Schistocerca americana*)

Ringer (.0018 *M* $CaCl_2$) mm./min.	Ca-free Ringer at 7 min. mm.	2.5 ×conc. Ca (.0044 *M*) in Ringer mm./min.	10 ×conc. Ca (.0175 *M*) in Ringer mm./min.	K oxalate (.005 *M*) in Ca-free Ringer after 15 min. mm.	Na oxalate (.005 *M*) in Ca-free Ringer after 15 min. mm.
1.281	0.536	1.616	2.315	0	0
1.144	0.526	1.498	2.143	0	0
1.053	1.135	1.798	2.433	0	0
0.962	0.490	1.398		0	0
1.262		1.253		0	0
0.944				0	0
0.726				0	0
1.088					
0.971					
1.050	0.671	1.512	2.297		

Figures show rate at one minute unless otherwise indicated.

centration normally present in insect Ringer) had an average rate of clotting of 1.050 mm./min. This relatively high rate of movement of the clot is not maintained during the later stages of the clotting process (see Fig. 2). Thus, the rate of clotting of fibers exposed to 0.0018 *M* $CaCl_2$ had an average clotting rate of 0.332 mm./min. at 5 minutes and 0.211 mm./min. at 10 minutes. Fibers exposed to 0.0044 *M* $CaCl_2$ showed an average rate of clotting of 0.394 mm./min. at 5 minutes and 0.234 mm./min. at 10 minutes. Those fibers immersed in 0.087 *M* $CaCl_2$ and those immersed in 0.0175 *M* $CaCl_2$ had an average rate of clotting of 0.596 mm./min. and 0.696 mm./min. at 5 minutes and 0.410 mm./min. and 0.425 mm./min. at 10 minutes, respectively.

The rate of movement of the protoplasmic clot of grasshopper muscle fibers in Ca-free Ringer is difficult to determine during the first few minutes of observation. These difficulties are encountered only during the first 6–7 minutes. This period is characterized by a gradual darkening of the protoplasm in the area adjacent to the cut or exposed end. There is no definite boundary separating the clotted re-

gion from the unclotted portion of the fiber. However, after 7 minutes, in all cases, there was a distinct clotted portion with a definite boundary characteristic of fibers exposed to higher concentrations of the calcium ion. In Figure 2 the time during which there is indistinct clotting is represented by a broken line. The average rate of clotting of 5 fibers at 7 minutes was 0.072 mm./min. and 0.066 mm./min. at 11 minutes.

b. Inhibition of clotting. It has been shown that the rate of movement of the protoplasmic clot in muscle fibers is dependent upon the calcium concentration. The question now arises as to whether or not the clot progresses in the absence of calcium.

1) Blue crab

With muscle fibers from the blue crab (*C. sapidus*), it was unnecessary to use sodium or potassium oxalate to prevent the movement of the protoplasmic clot. Fibers dissected and cut in Ca-free sea water and allowed to remain in this medium for periods of from 20–36 minutes showed no evidence of protoplasmic clotting

TABLE IV

Effects of calcium on rate of protoplasmic clotting (single muscle fibers)

Blue Crab (*Callinectes sapidus*)

Artificial sea water (0.0117 *M* $CaCl_2$) mm./min.	Isotonic $CaCl_2$ (.33 *M*) mm./min.	1/2 isotonic $CaCl_2$ (.165 *M*) mm./min.	Ca-free sea water (from 20–36 min.) mm./min.
0.287	0.586	0.448	0
0.362	0.513	0.456	0
0.382	0.578	0.619	0
0.225	0.448	0.464	0
0.327	0.741	0.619	0
0.265	0.448	0.448	0
0.205	0.432	0.456	0
	0.603	0.513	0
	0.472	0.554	0
0.293	0.534	0.508	

during this period of exposure (see Table IV). Upon return to artificial sea water containing a concentration of calcium of 0.11 *M*, these same fibers immediately showed the clotting reaction characteristic of muscle exposed to calcium.

2) Grasshopper

In the absence of calcium from the surrounding medium, the protoplasmic clot progresses. Thus, if a single muscle fiber from the grasshopper is isolated in Ca-free insect Ringer, a protoplasmic clot will form and progress (see bottom curve, Fig. 2). However, either sodium or potassium oxalate when mixed with Ca-free insect Ringer is effective in preventing the formation of a clot (see Table III).

The concentration of potassium (or sodium) oxalate which was most effective with grasshopper muscle fibers was 0.005 *M*. Higher concentrations usually resulted in death of the muscle fibers, whereas lower concentrations were not effective in preventing the movement of the clot. Figure 3 summarizes the results of experiments with potassium oxalate in inhibiting the rate of movement of the proto-

plasmic clot. A series of experiments was performed in which muscle fibers of the grasshopper (*S. americana*) were cut in a K-oxalate (0.005 *M*)-Ca-free insect Ringer solution and allowed to remain in this solution for 15 minutes. During this period, no protoplasmic clot could be observed in these fibers (see bottom curve, Fig. 3). However, after the 15-minute period had expired, each muscle fiber was placed in normal insect Ringer. There was an immediate clotting of the muscle protoplasm. The rate of movement of this protoplasmic clot was 1.245 mm./min. during the first minute and 2.040 mm./min. at the end of 5 minutes (average of 5

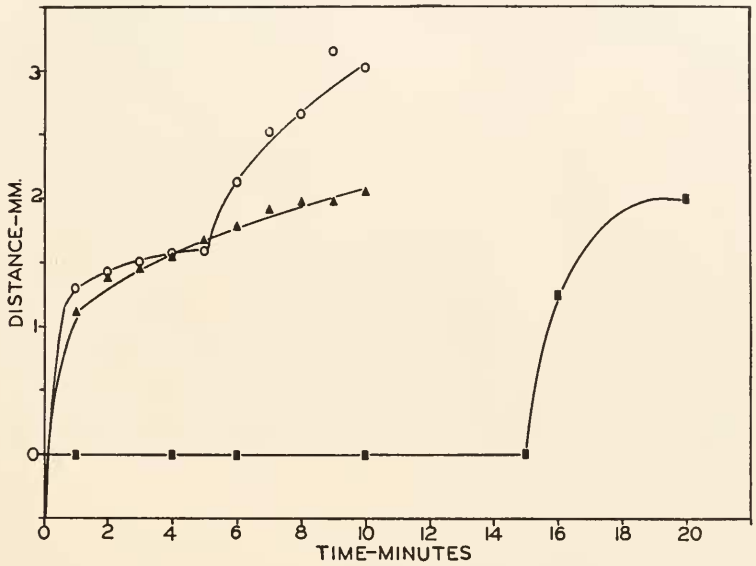

FIGURE 3. The effects of oxalate on protoplasmic clotting in single muscle fibers of the grasshopper (*Schistocerca americana*). Legend: Open circles (○), Ringer for one minute, K-oxalate (0.005 *M*) for the next 4 minutes, Ringer for 5 minutes, average of 6 fibers; triangles (▲), control, Ringer for 10 minutes, average of 5 fibers; squares (■), K-oxalate (0.005 *M*) for 15 minutes, Ringer for 5 minutes, average of 5 fibers.

fibers). These experiments clearly demonstrate the inhibitory effect of K-oxalate on the movement of the clot in muscle protoplasm. The effectiveness of both potassium and sodium oxalate is shown in Table III.

Another experiment was performed to determine whether this clotting reaction, once initiated by calcium, could be stopped. In this experiment a muscle fiber was first exposed to normal insect Ringer (containing calcium as the clotting agent) for a period of time sufficient to initiate the clotting reaction. Then the fiber was washed and the Ringer solution replaced with an oxalate-Ringer solution. After observations were made on the progress of the clot, the oxalate-Ringer solution was replaced with normal insect Ringer and the rate of clotting was again observed. The results of this experiment are shown in Figure 3 (top curve). A detailed account of this experiment follows. Muscle fibers were cut and allowed to remain in normal insect Ringer (calcium concentration, 0.0018 *M*) for one minute (see Fig. 3, top curve). During this period the average (6 fibers) rate of movement

of the clot was 1.300 mm./min. These fibers were then quickly washed three times with oxalate-Ringer (0.005 *M* K-oxalate in Ca-free insect Ringer) and allowed to remain in this solution for four minutes. The average rate of movement of the clot of these 6 fibers was determined at one-minute intervals during exposure to the oxalate-Ringer solution. In Figure 3 (top curve) the point at one minute indicates the distance the clot had progressed during a one-minute exposure to normal insect Ringer. The next four points (2, 3, 4 and 5 minutes) indicate the progress of the clot under the influence of oxalate-Ringer for 4 minutes. The average rates of movement of the protoplasmic clot while immersed in oxalate-Ringer were: 1.432 mm./min. during the first minute; 0.750 mm./min. during the second minute; 0.525 mm./min. for the third minute; and 0.936 mm./min. during the fourth minute. These fibers were then washed with normal insect Ringer and the rate of movement of the protoplasmic clot was determined at one minute intervals in insect Ringer. As indicated by the curve (Fig. 3, top curve), exposure of these previously oxalated fibers to a solution containing calcium (normal insect Ringer) results in a rapid increase in the rate of movement of the protoplasmic clot. The average rates of movement of the clot during a 5-minute exposure to normal insect Ringer were: 2.147 mm./min., 1.261 mm./min., 0.888 mm./min., 0.789 mm./min., and 0.606 mm./min., respectively.

Six control fibers, cut and allowed to remain in normal insect Ringer for 10 minutes, gave the following average rates of movement of the clot: during the first minute, 1.071 mm./min.; second minute, 0.673 mm./min.; third minute, 0.483 mm./min.; fourth minute, 0.396 mm./min.; fifth minute, 0.339 mm./min.; sixth minute, 0.302 mm./min.; seventh minute, 0.275 mm./min.; eighth minute, 0.243 mm./min.; ninth minute, 0.229 mm./min.; and tenth minute, 0.214 mm./min. (see triangles, middle curve, Fig. 3).

Discussion

Of the four major cations (Na^+, K^+, Mg^{++} and Ca^{++}) normally present in protoplasm, the calcium ion has long been known to be of primary importance in physiological processes. Its importance has been demonstrated in the clotting of blood and milk, the surface precipitation reaction of various cells, and more recently in relation to the stimulation in muscle. In fact, Heilbrunn has proposed a theory of stimulation and anesthesia based on the presence of calcium in the cortex of the cell and its subsequent movement to the interior. For a discussion of this theory and the relation of calcium to these phenomena see Heilbrunn (1943) and Woodward (1948). In order to study the effect of the calcium ion on muscle, physiologists have used isolated muscle fibers of vertebrates. The data in this paper reveal some of the similarities between vertebrate and invertebrate muscle fibers in their response to calcium.

One of the first visible effects of the calcium ion on vertebrate (frog) muscle fibers is a marked shortening of the fiber (Heilbrunn, 1940). In studies on isolated muscle fibers of three arthropods (blue crab, lobster and grasshopper) it was found that generally these forms follow the same pattern as vertebrate isolated muscle fibers. In the case of the lobster and the blue crab the fibers shortened to a lesser degree than isolated fibers from the frog in comparable solutions of calcium chloride. Thus, with frog fibers Heilbrunn found an average shortening of 73%.

The average percentage of shortening in muscle fibers of the blue crab was about 45% and in fibers of the lobster about 34%. Normal insect Ringer with a calcium concentration of 0.0018 *M* caused an average percentage of shortening of 58% in grasshopper muscle fibers. The evidence presented indicates that the sensitivity of muscle fibers of these forms to calcium is dependent upon the amount of calcium normally present in their environment.

A second visible effect of calcium on arthropod muscle fibers was the clotting reaction reported earlier by Speidel (1938), Heilbrunn and Wiercinski (1947) and Woodward (1948). The data show that this protoplasmic clotting in arthropod muscle is quite similar to that found by other investigators in vertebrate (frog) muscle fibers. However, the rate at which the movement of the protoplasmic clot occurs in the grasshopper indicates that the muscles of this animal are much more sensitive to low concentrations of calcium than those of the frog or the blue crab. Electrical stimulation has been used as a criterion for determining whether single muscle fibers of the frog are viable. With arthropod single muscle fibers, this method of determining their viability was found to be unreliable. A convenient indication of the viability of these fibers is the effect produced by the calcium ion—the clotting reaction.

It is obvious, therefore, that the effect of calcium on muscle protoplasm is much the same for muscle fibers of widely different types of animals. In frog muscle, however, it is difficult or impossible to prevent shortening and the clotting reaction by removing calcium from the surrounding medium. In all the invertebrate muscles I have studied, removal of calcium from the surrounding medium prevents shortening and clotting. This fact adds additional support to Heilbrunn's point of view, for it shows a complete dependence of this clotting of muscle protoplasm on the calcium ion.

Experiments showed that, once initiated, this clotting reaction, particularly in the grasshopper, does not stop immediately. There is a gradual decrease in the speed of movement of the clot in oxalate-Ringer solution. The data presented (Fig. 3) show a significant difference between the rate of movement of the clot in oxalate-Ringer solution as compared to normal insect Ringer. This suggests that not only is this clotting reaction a calcium-initiated phenomenon, but also that it requires calcium for its continuance.

Summary

1. When isolated single muscle fibers of blue crab, lobster and grasshopper have their cut ends exposed to solutions containing the calcium ion, they show the same effects as do frog muscle fibers; that is to say, they shorten and the protoplasm clots. However, the wave of clotting is faster in grasshopper muscle than it is in frog muscle. The degree of shortening of arthropod muscle fibers is less than that of frog muscle.

2. Evidence indicates that single muscle fibers of the grasshopper are more sensitive to calcium ions than are single fibers of the frog and blue crab.

3. The rate of movement of the protoplasmic clot in invertebrates is relatively rapid during the first minute. However, there is a gradual decrease in this rate after the first minute.

4. The clotting reaction can be prevented in invertebrate muscle fibers. In grasshopper muscle fibers, concentrations of 0.005 *M* sodium or potassium oxalate are effective in preventing the formation of the protoplasmic clot. Ca-free sea water is in itself sufficient to prevent the formation of the protoplasmic clot in muscle fibers of the blue crab. Once initiated, this clotting reaction in crab muscle cannot be stopped immediately by the application of sodium or potassium oxalate.

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